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14. ABSTRACT
The long term goal of this work is to develop a new prognostic tool with which to determine the response of a patient to a given therapy, with the view of providing the most appropriate treatments tailored to individual patients. The central hypothesis of this proposal is that a subset of the genes expressed in a prostate tumor can be used to predict response to specific therapeutic regimens. The purpose of this work is to generate predictive methods which will allow patients to be selected for specific treatment protocols. In this year, per our proposed schedule, we have continued to focus on acquisition of tissue samples and their grafting and treatment in SCID mouse hosts. We have now collected a total of 136 out of a required 150 human prostate cancer sample sets. We have tested and validated methods for amplifying RNA from human prostate tissues grafted to SCID mouse hosts and have run a series of experiments to optimize the microarray technology for these samples. This has included developmental in silico bioinformatics work to standardize the signal intensity between samples making comparisons meaningful. RNA has been prepared for amplification and microarray analysis of the first 136 samples is underway. The project is proceeding behind to its predicted timeline due to delays in collection of human tissues.

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DAMD 17-03-1-0047

Therapy selection by gene profiling

P.I. Simon W. Hayward, PhD

Introduction

The **long-term goal** of this work is to develop a new prognostic tool with which to determine the response of a patient to a given therapy, with the view of providing the most appropriate treatments tailored to individual patients. The **central hypothesis** of this proposal is that a subset of the genes expressed in a prostate tumor can be used to predict response to specific therapeutic regimens. The **purpose** of this work is to generate predictive methods that will allow patients to be selected for specific treatment protocols. A subordinate aim is to catalogue genes that are regulated in response to treatment with Taxotere, in both responding and non-responding human prostate cancer tissue samples, since these genes might suggest additional targets for therapeutic intervention. The **rationale** is to utilize a novel method of tissue grafting in combination with state of the art microarray, biostatistical and bioanalytical analysis to generate new prognostic tools. This project is an essential "proof of principle" step in the sense that if this methodology is successful with Taxotere it should be applicable to any new therapeutic approach that exists or which will be developed in the future. This project is divided into four specific aims. The first three aims will primarily generate data on the predictive value of gene expression profiles in samples derived from patients in determining the response of those patients to treatment with Taxotere. This work will allow us to design new predictive microarray or multiplex real time reverse transcriptase polymerase chain reaction (RT-PCR) assays to determine whether specific patients will respond to Taxotere. The fourth specific aim will use existing samples from patients engaged in an ongoing clinical trial to test whether these predictions are valid in a clinical setting.

Original Statement of Work

Therapy selection by gene profiling.

Task 1

Generate deoxyribose nucleic acid (DNA) Microarray patterns for prostate cancer samples from 150 patient tumor samples

a) As cases present, collect 150 histopathologically-confirmed prostate cancer tissue cores. Snap freeze core fragments (months 1-24) **Current status: Modified per first annual report because of changes in clinical practice to grafting of samples into untreated mice (also for Task 1b). 136 of the 150 samples have been processed. Collection of the final 14 samples is ongoing.**

b) Prepare ribose nucleic acid (RNA) from snap frozen core fragments (months 1-25) **Current status: Completed for 136 samples.**

c) Run 150 comparative DNA microarrays using 12k human chip against a mixed sample human prostate standard (months 1-25). **Current status: First 136 samples undergoing microarray analysis on 30k oligonucleotide chips.**

Task 2.

In vivo studies

a) Perform preliminary study to determine optimal post-treatment timepoint for determining histopathological response to Taxotere (months 1-3). **Current status: Completed in year 1.**

b) Graft tissues from the cores used in task 1 to pairs of severe combined immune-deficient (SCID) mice (months 1-24). **Current status: Completed for 136 samples, ongoing to collect a total of 150.**

c) After 30 days treat one of each pair of mice with Taxotere for 6 days (months 1-25). **Current status: Completed for 136 samples, ongoing to collect a total of 150.**

d) Sacrifice mice and harvest tissues. Snap freeze tissues, make RNA for microarray analysis, take representative tissue samples for histology (months 2-26). **Current status: Completed for 136 samples, ongoing to collect a total of 150.**

e) Run 300 comparative microarrays of untreated vs standard and Taxotere-treated vs standard samples (months 3-28). **Current Status: Initiated.**

This task requires the use of 195 male SCID mice

Task 3
Biostatistical analysis

a) Identification of gene expression patterns which predict histopathologic response to Taxotere. Biostatistical analysis to determine a pattern of gene expression in tissue cores which predict histopathologic response to Taxotere in a xenograft model (months 26-32). **Current Status: Not yet initiated.**

b) Identification of genes regulated by Taxotere in responsive and non-responsive tissues. Biostatistical and bioinformatic analysis will be used to identify genes regulated by Taxotere in responsive and non-responsive tissue samples (months 28-34). **Current Status: Not yet initiated.**

c) Design of assays microarrays to predict response to Taxotere. Custom microarrays or assays (depending upon the number of prognostic genes identified in 3a) will be designed in which expression patterns of a limited number of genes should predict the response of human prostate cancer to Taxotere (months 32-35). **Current Status: Not yet initiated.**

Task 4

Prediction of response of patients in a clinical trial setting (months 35-36) **Current Status: Not yet initiated.**

Based upon microarray analysis of archived snap frozen tissue the ability of the arrays designed in task 3 to predict response in a clinical trial will be tested. Prepare RNA from archived tissue cores. Run microarrays, predict response based upon data acquired in earlier tasks. Test results by breaking patient code and correlating actual and predicted responses.

Work Ongoing and Completed

The main tasks for the second year of this award have been, 1) to continue to collect and graft patient-matched pairs of human prostate cancer tissue samples into SCID mice as xenografts, and 2) to treat one mouse from each pair with Taxotere, and subsequently (six days post-treatment) to harvest the tissues from both the control and treated mouse for analysis.

It is noted that the pilot study which constitutes task 2a of the statement of work (SOW) was completed at the initiation of this project. The results confirmed the preliminary studies in the initial proposal suggesting that day six post treatment was the most effective time point to focus upon apoptosis resulting from Taxotere treatment using TdT (terminal deoxynucleotidyl transferase)-mediated dUTP nick end labeling (TUNEL) analysis as a measure.

At the time of writing this amended report the numbers of sample sets (pairs of patient-matched xenografted control and Taxotere-treated tumor samples) has now increased to a total of 136 out of a target of 150. Control and Taxotere-treated xenografts have been subdivided with fractions fixed in formalin, snap frozen and preserved in RNA later.

The formalin-fixed Taxotere-treated samples have been processed to paraffin, sectioned and stained using both hematoxylin and eosin and TUNEL. Results have been recorded as digital images for analysis. TUNEL staining is presently being quantitated to provide a quantitative measure of response to Taxotere for each patient sample. This information will be used during the analysis of the microarray data.

To provide a second level of assessment of response to Taxotere we have entered into a collaboration with the Wang laboratory at the British Columbia Cancer Agency in Vancouver to provide an independent sorting of the Taxotere response based upon histologic criteria which Dr. Wang's group have been developing for xenografted human tissues. This analysis will be performed independently of the apoptotic staining study. The staff involved in the two forms of analysis will be blinded to each other's results. This should give us additional discriminatory power when assessing the response status of each sample and will allow an independent assessment of the two methods of analysis.

In order to proceed with this project it was necessary to run pilot tests to determine the suitability of the chosen molecular analytical platforms. Therefore the amplification and microarray platforms have been validated for xenografted human prostate samples. As shown in figure 1 the RNA prepared from these xenografts can be successfully amplified. A successful amplification is one judged to have the majority of the products sized above 150. this has been consistently achieved.

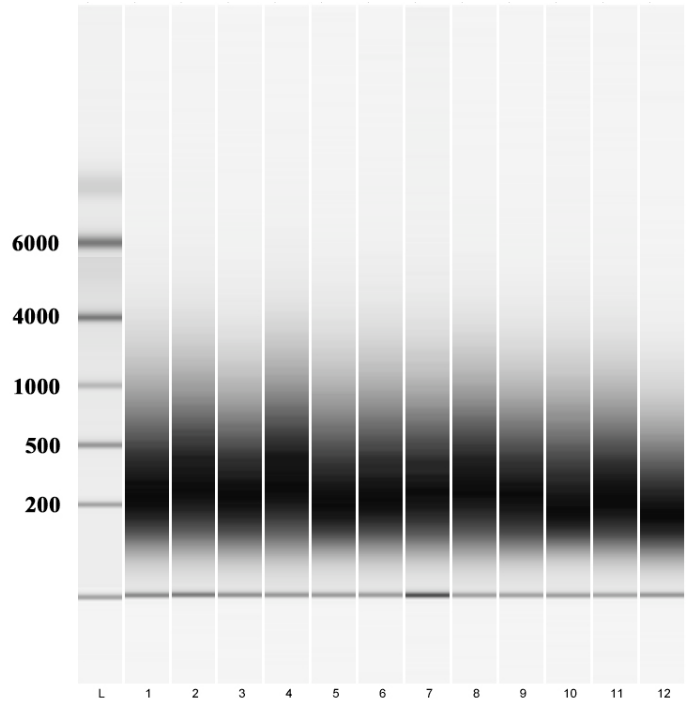

Figure1. Size of amplification products from human prostate tissue xenografts to SCID mice.

The change in microarray format noted in the first annual report to a 30k human oligo array resulted in technical difficulties for the microarray core. The core physically moved locations resulting in damage to a printing robot and then encountered technical difficulties with the arrays that were solved by changing to a mirrored slide format. This format now works extremely well and we have been able to perform test runs using RNA prepared from xenografted human prostate samples. A certain amount of bioinformatic troubleshooting by Ms Boone and Dr. Levy in the Vanderbilt Microarray Shared Resource was required to mathematically center the data sets to allow comparisons to proceed. This has now been completed and we can successfully examine and compare data from xenografted human prostate tissue. It should be noted that this delay has not materially affected progress in the overall project since the limiting factor has been the rate of acquisition of patient tissue samples, discussed below.

As noted in the first annual report Dr. Shawn Levy director of the Vanderbilt Microarray Shared Resource has requested that we perform microarray analyses in batches of 48 or 96 as this allows each batch to be performed with a single lot of reagents maximizing internal consistency. RNA has been prepared from the initial 136 sample sets and this aspect of the work is now underway.

Collection of tumor samples has been slower than initially predicted resulting in a delay in moving to the initial data analysis section of the work. This is a factor over which we have no control. The decrease in the number of samples available from the expected level is largely a function of the limited amounts of tumor tissue now found in patients. Due to increased surveillance tumors are detected and patients are undergoing surgery earlier in the course of their disease. This means that the tumor sizes are often very small. Thus we now commonly receive tissue cores which upon, histopathologic analysis, are found not to contain tumor tissue and which cannot therefore be used for this study. There has been no diminution in either the numbers of patients or the numbers of surgeries, but rather in the likelihood that any given surgery will provide tumor tissues useful for our purposes.

Technical modifications:

Technical modifications relating to changes in clinical practice which has affected tumor tissue collection and changes in the microarray format from that originally proposed were noted in the first annual report. No significant changes have occurred in the current year.

Personnel Changes

As noted in the first annual report we have had a number of personnel changes in the Department of Pathology resulting in tissue acquisition and processing passing to the Vanderbilt-Ingram Cancer Center tissue Acquisition Core under the leadership of Dr. Kay Washington. This is a fee for service arrangement which has worked well over the last year. Since the last report Dr. Richard Roberts has left Vanderbilt and has been replaced by another genitourinary pathologist Dr. Marcia Wills. This is effectively a neutral change in regard to this project but is noted here in the interests of complete reporting.

Key Research Accomplishments

- The methods for generating and amplifying RNA from xenografted human prostate tumors at a quality suitable for microarray analysis have been tested and validated.
- The microarrays have been tested and initial troubleshooting to validate the use of this specific microarray format for these samples has been successfully performed.

Reportable Outcomes.

None

Conclusions.

This work is behind the predicted timeline, the reason for this is rate of acquisition of human prostate cancer tissue samples which has been lower than expected. This is largely a product of the ability to detect disease at progressively earlier stages and the consequent reduction in tumor tissue available for research. For this reason we will be applying for a no cost extension to complete the proposed work. There are no significant deviations from the original SOW beyond the operational changes (in microarray format and processing of tissue resulting from clinical use of robotic surgical technique) noted in the previous report. The methods to be used in the next phase of the work have been tested and validated.